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Quantification of the damage caused by *Meloidogyne enterolobii* in okra

Abstract – The objective of this work was to estimate the damage caused by *Meloidogyne enterolobii* in okra (*Abelmoschus esculentus*) and to verify the reliability of the reproduction factor as a suitable measure for selecting resistant okra genotypes. Increasing populations of *M. enterolobii* – 0, 500, 1,500, 3,000, 5,000, and 7,000 eggs and second-stage juveniles (J_2) per plant, – were evaluated, in a completely randomized design. The pathogen showed a parasitism pattern similar to that of *M. incognita*, causing a significant decrease in morphological and agronomic traits. The pathogen reproduction factor should be used in the selection of okra genotypes for tolerance to *M. enterolobii*, in populations above 3,000 eggs or J_2.

Index terms: *Abelmoschus esculentus*, reproduction factor, root-knot nematode, susceptible host.

Quantificação do dano causado por *Meloidogyne enterolobii* em quiabeiro

Resumo – O objetivo deste trabalho foi estimar os danos causados por *Meloidogyne enterolobii* em quiabeiro (*Abelmoschus esculentus*) e verificar a confiabilidade do fator de reprodução como medição adequada para a seleção de genótipos de quiabeiro resistentes. Avaliaram-se populações crescentes de *M. enterolobii* – 0, 500, 1.500, 3.000, 5.000 e 7.000 ovos e juvenis de segundo estádio (J_2) por planta –, em um delineamento inteiramente casualizado. O patógeno apresentou padrão de parasitismo similar ao de *M. incognita*, tendo causado redução significativa de parâmetros morfológicos e agronômicos. O fator de reprodução do patógeno deve ser usado na seleção de genótipos de quiabeiro quanto à tolerância a *M. enterolobii*, em populações acima de 3.000 ovos ou J_2.

Termos para indexação: *Abelmoschus esculentus*, fator de reprodução, nematódeo-da-galha, hospedeiro suscetível.

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Okra – *Abelmoschus esculentus* (L.) Moench – production is strongly influenced by root-knot nematodes (*Meloidogyne* spp.) (Marin et al., 2017). These plant parasites cause damage that leads to a reduced vegetative development and, consequently, to a lower yield (Mukhtar et al., 2017).

Although less frequently reported as occurring in okra cultivations, *Meloidogyne enterolobii* (*M. mayaguensis*) (Yang & Eisenback, 1983) has gained importance in Brazil and worldwide, as this species is not controlled by the same sources of resistance as other *Meloidogyne* species (Carneiro et al., 2012). Moreover, the widespread cultivation of plants resistant to other root-knot nematode species can create a selection pressure in favor of *M. enterolobii*, increasing the importance of this species in vegetable crops (Diniz et al., 2016).

The effect of different *Meloidogyne* species on okra, especially *M. incognita*, has been widely studied, but studies addressing the quantification of the impact of *M. enterolobii* on okra are lacking.

The objective of this work was to estimate the damage caused by increasing populations of *M. enterolobii* on okra, and to verify if the reproduction factor is a reliable measure for the selection of okra genotypes resistant to the pathogen.

The experiment was carried out from September 2016 to January 2017, in a controlled environment in the Universidade Estadual Paulista (Unesp), in Jaboticabal – at 21º14'05"S, 48º17'09"W, 614 m altitude), SP, Brazil. According to the classification of Köppen & Geiger (1928), the climate is type Aw (tropical with dry winter and excessive rains), in transition to Cwa (subtropical climate with warm dry winter).

Evaluation were performed in the initial populations (IP) for increasing densities of eggs at 0 (inoculation with water), 500, 1,500, 3,000, 5,000, and 7,000 eggs, or for second-stage juveniles (J_2) per plant. Okra 'Santa Cruz 47' was chosen as host, as it is the most commonly okra cultivar planted in Brazil and it is a highly susceptible to common root-knot nematode species. Seedlings were produced in 128-cell expanded polystyrene trays, filled with with coconut fiber-based substrate. After sowing, the trays were conditioned in a greenhouse equipped with a sprinkler irrigation system. Transplanting was performed 15 days after sowing in 13 L plastic pots filled with a mixture of soil, sand, and tanned bovine manure at 3:1:1, which had been autoclaved (120ºC, 1 atm, 1 hour).

The inoculation of egg and J_2 was performed during transplanting, using a digital pipette. The inocula were extracted from 'Paluma' guava (*Psidium guajava* L.) roots, according to the method of Hussey & Barker (1973). The identity of inocula was confirmed by the nematology laboratory at Unesp, based on the original description of the species (Yang & Eisenback, 1983).

We evaluated the vegetative development and agronomic characteristics of okra, besides the nematode reproduction. Two or three harvests were made per week, according to fruit development, to measure the number of fruit and the yield. At the end of the experiment, we measured the plant height, stem diameter at plant base, fresh shoot mass, and fresh root mass in 10 plants per plot. In order to estimate the nematode reproduction, the roots were weighed and then processed according to Hussey & Barker (1973). The final population was estimated using a Peters' chamber under a photonic microscope (Quimis Aparelhos Científicos Ltda., Diadema, SP, Brazil). The reproduction factor (RF) was calculated by dividing the final population (FP) by the initial population (IP).

The data were transformed using $(x+5)^{0.5}$. The analysis of variance was performed, and the means were compared by Tukey's test, at 5% probability, using the AgroEstat statistical software (Barbosa & Maldonado Júnior, 2015).

The greatest final population (FP) means was observed in the infestation at the highest-population density (Table 1); while the highest reproduction factor (RF) mean was observed for the lowest-population density. This indicates that caution should be taken in the use of RF to select resistant genotypes, since, although higher-population densities result in larger final populations, the reproduction does not occur at sufficient rates to result in RF coherence. Experiments aiming at screening okra genotypes resistant to root-knot nematodes should inoculate fewer than 3,000 eggs and J_2 juveniles, so that there is no overestimation of the actual plant host status. At least, RF should be used considering more than one selection parameter instead of it alone.

The highest-reproduction rate observed at the lowest-population densities may be related to the

Table 1. Means of the final population of eggs and second-stage juveniles, and reproduction factor of *Meloidogyne enterolobii* populations in okra 'Santa Cruz 47'[(1)].

Initial population	Final population	Reproduction factor
0	0.0a	0.0a
500	519.2b	1038.4c
1500	872.0b	581.4bc
3000	848.2b	282.8b
5000	1861.4c	372.2b
7000	2033.2c	290.5b
Coefficient of variation (%)	29.48	37.30

[(1)]Means followed by equal letters do not differ by Tukey's test, at 5% probability. Data were transformed using $(x+5)^{0.5}$, before performing the statistical analysis.

higher-food availability to the nematodes (Mahalik & Sahoo, 2016). However, a lower nematode number per root may indicate a host-tolerant population level.

The presence of root-knot nematodes can lead to root system destruction and, at high populations, nematodes that have already parasitized the roots may compete for food, resulting in the possibility that juveniles of subsequent generations may not have new infection sites.

There was a significant reduction of plant height and fresh shoot mass in the treatments inoculated with *M. enterolobii*, in comparison to the control treatment without inoculation (Table 2). Similar negative effects were reported by Mukhtar et al. (2013, 2014) and Hussain et al. (2016a) studying other root-knot nematodes.

No difference was observed between the treatments for stem diameter at the plant base and in natura fruit mass (Table 2). Therefore, these are traits of little interest for estimating the damages caused by *M. enterolobii* in okra.

The fresh matter mass of roots was higher in treatments inoculated with *M. enterolobii* (Table 2). Similar results were found for *M. incognita* by Mukhtar et al. (2013, 2014), and Hussain et al. (2016b).This is probably due to fact that the presence of nematodes in the root system forms galls through hyperplasia and hypertrophy, contributing to a greater fresh matter mass.

Significant differences were observed for the number of fruit and yield per plant (Table 2). The highest mean of these traits was observed for the control (without inoculation), while the lowest mean was observed in the treatment with 7,000 eggs and J_2 per plant. Neither yield nor number of fruit was influenced with populations up to 3,000 eggs and J_2 per plant; therefore, for low initial populations, the reduction of production is not significant.

The reduction of production parameters may be related to the injuries caused to the root during penetration, or to the nematode feeding (Mukhtar et al., 2017). The upward movement of water and nutrients is commonly impaired with nematode infection, as the roots have their permeability to water reduced. The morphological changes that occur after infection by *Meloidogyne* spp. may also alter the distribution of photoassimilates, leading the infected roots to drain more than the shoot, which directly influences the production (Hussain et al., 2016a).

Even though treatments with greater population densities showed a lower RF, the greatest negative effects of the infestation to yield were observed in these conditions, probably because of the higher-final population of nematodes. Thus, in screening experiments of okra genotypes, both for resistance and tolerance, the RF should only be used for the initial populations, above 3,000 eggs and J_2. In smaller initial populations, there is a tendency to overestimate the reproduction factor.

The nematode *M. enterolobii* shows a pattern of parasitism similar to that of *M. incognita*, causing a significant harm to morphological and agronomic traits.

Table 2. Effect of initial populations of *Meloidogyne enterolobii* in the following okra 'Santa Cruz 47' traits: plant height, stem diameter at plant base, fresh matter mass of shoot and roots, number of fruit, average fruit mass (in natura), and yield per plant[(1)].

Initial population	Plant height (cm)		Stem diameter (mm)		Shoot fresh matter mass (kg)		Root fresh matter mass (kg)		Number of fruit		Fruit mass (g)		Yield (kg per plant)	
	Mean	RR (%)	Mean	RR (%)	Mean	RR (%)	Mean	RR (%)	Mean	RR (%)	Mean	RR (%)	Mean	RR (%)
0	124.7a	0.0	1.50	11.24	110.5a	0.0	65.5a	53.71	9.8a	0.0	20.43	0.0	0.199a	0.0
500	107.6ab	13.71	1.57	7.10	94.0ab	14.93	89.0a	37.10	8.2ab	16.33	20.01	2.60	0.164ab	17.59
1,500	105.3ab	15.56	1.54	8.80	95.0ab	14.09	96.7ab	31.66	8.0ab	18.37	20.10	1.62	0.157ab	21.11
3,000	101.8b	18.76	1.47	13.02	100.5ab	9.05	102.0ab	27.92	6.4b	34.69	19.34	5.34	0.124b	34.49
5,000	101.7b	18.44	1.61	4.94	84.5ab	23.25	91.5a	35.34	7.8ab	20,41	18.15	11.16	0.142b	28.64
7,000	101.3b	18.77	1.69	0	80.0b	27.70	141.5b	0	6.2b	36.73	20.20	1.13	0.126b	36.68
CV (%)	6.64		1.37		10.67		16.75		22.31		15.41		25.52	

[(1)]Means followed by equal letters do not differ by Tukey's test, at 5% probability. Data were transformed using $(x+5)^{0.5}$, before performing the statistical analysis. RR, relative reduction. CV, coefficient of variation.

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